lined through in pencil on the form PTO-1449. The copy of the PTO-1449 enclosed with the present Office Action shows that all of the references cited thereon, except FR2128587 and EP0596812, were initialed by the Examiner.

Applicants respectfully request confirmation from the Examiner in the next Office Action that the initialed references on the PTO-1449 were considered by the Examiner. Applicants also bring to the Examiner's attention that EP0596812 has two English language counterparts, U.S. Patent No. 5,629,190 and U.S. Patent No. 5,635,391. U.S. Patent No. 5,635,391 was cited by the Examiner in the present Office Action.

At page 3 of the Office Action, the Examiner rejected claims 21-43 under 35 USC 112, first paragraph, as not enabled. The Examiner indicated that claim 21 is drawn to an industrial process for expressing heterologous proteins in *E. coli* W host cells, but the specification discloses only systems based on pRPA-BCAT4 that under specific conditions produce high levels of nitrilase which is insufficient to put one of skill in the art in possession of the attributes and features of all species within the claimed genus.

Applicants traverse this rejection. Claim 21 has been amended to state that the suitable system for expressing heterologous proteins comprises a P_{trp} promoter. Claims 22-37 and 39-42 depend from claim 21 and are also amended by the foregoing amendment to claim 21. In claim 43, which is directed to an *E. coli* strain W host cell, the suitable system for expressing heterologous proteins comprises the P_{trp} promoter. The P_{trp} promoter has been used, as shown in the examples, for expression of heterologous proteins in *E. coli* strain W. Withdrawal of this section 112, first paragraph is requested.

The Examiner provisionally rejected claims 21-38 and 40-43 under 35 USC 112, first paragraph as not enabled because it is not apparent that plasmid pRPA-BCAT4 and derivative plasmids are readily available to the public.

Applicants also traverse this rejection. Plasmid pRPA-BCAT4 is described in French patent application FR 96/13077, which issued in the United States as U.S. Patent No. 6,180,359, in Example 2 and Figure 3. A copy of U.S. Patent 6,180,359 is submitted for the Examiner's attention. Additionally, the expression cassette represented by SEQ ID NO: 2 shows the sequence of the nitB gene wherein amino acid 279 has been changed

from Asp to Asn. Plasmid pRPA-BCAT4 and derivative plasmids are readily available to the public. Withdrawal of this section 112, first paragraph rejection is requested.

At page 6 of the Office Action, the Examiner rejected claims 21-44 under 35 USC 112, second paragraph as indefinite.

The Examiner objected to the term "industrial process" in claim 1 stating that it is unclear what is the essential property that makes the process "industrial", and that the specification defines the term at page 10, lines 4-14 by non-limiting examples.

The term "industrial process" is defined in the specification and is not indefinite. The term 'industrial process" is defined in the specification at page 10, lines 4-14 as any process in which the bacterial culture volume is greater than the culture volume employed in research laboratories, and that, generally, the term is intended to mean any process for which the culture volume is greater than two liters. It is thus the volume of the bacterial culture that makes the process industrial.

Also in claim 21, the Examiner stated that usually the words expressing "in" not "into" *E. coli* are used. In claim 21, the first step is introducing a suitable system for expressing heterologous proteins into an *E. coli* bacteria strain W host cell. The term "into" correlates with "introducing". This is the customary use of the word and is therefore not indefinite.

The Examiner objected to the term "high" in claim 24, stating that it is a relative term which renders the claim indefinite because the claim does not define "high", the specification does not provide a standard for ascertaining the requisite degree and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention.

The use of the term "high" in claim 24 is not unclear. Claim 24 is drawn to the method of claim 21, wherein the suitable culture medium is a culture medium suitable for high density of biomass and a high content of heterologous protein produced. Persons of ordinary skill in the art are reasonably apprised of the meaning of "high" with respect to *E. coli* culture media as used in claim 24. Page 6, lines 14-25 of the specification discloses that for the production of heterologous proteins in bacteria, the productivity of the culture system employed can be significantly increased by using high cell density culturing strategies and refers to Makrides *et al.* Microbiological Reviews 60: 512-538,

1996. Makrides *et al.*, which was cited by the Examiner in the present Office Action, is entitled "Strategies for Achieving High-Level Expression Of Genes in Escherichia coli". This publication generally discusses high density cell-culture systems and conditions at pages 525 and 526. Additionally, the specification discloses at page 10, lines 15-20 that several types of media can be used for production of high density of biomass and a high content of heterologous proteins produced and refers to Lee *et al.* Trends in Biotechnology 14: 98-105 (1996). Thus, persons of ordinary skill in the art understand the term "high" as used in claim 24 in relation to high density cell-culture media and high content of heterologous proteins produced without reference in the specification to a standard for ascertaining the requisite degree. The term "high" in claim 24 is therefore not indefinite.

The Examiner stated that the claims recite "*E. coli*" bacteria strain W host cell where it appears that "bacterial" or "bacterium" is intended. The Examiner further indicated that either term is redundant. Claims 21, 22, 23, 39, 43, and 44 have been amended to remove "bacteria".

The Examiner stated that with regard to claim 38 it is unclear which molecules in addition to the sequence of SEQ ID NO: 1 are encompassed by the term "P_{trp}". Claim 38 has been canceled without prejudice and its limitation that the suitable system for expressing heterologous proteins comprises a P_{trp} promoter has been added to claim 21. The specification discusses the P_{trp} promoter at page 13, lines 10-24 and page 14, lines 1 and 2.

SEQ ID NO: 1 is comprised of the P_{trp} promoter and the ribosome binding site of the λ phage cII gene. The plasmids constructed in Example 1 have the P_{trp} promoter linked to the ribosome binding site of the λ phage cII gene (RBScII), which is in turn linked to the nitB gene. The expression cassette in the plasmids constructed in Example 1 has the sequence of SEQ ID NO: 2. SEQ ID NO: 1 has the same sequence as bases 1-121 of SEQ ID NO: 2. Bases 1-121 of SEQ ID NO: 2 comprise the P_{trp} promoter and the ribosome binding site of the λ phage cII gene.

In accordance with the specification at page 13, lines 3-9, the suitable system for expressing heterologous proteins comprises regulation elements such as promoters,

ribosome binding sites and transcription terminators. Thus, the P_{trp} promoter can be combined with a ribosome binding site, such as the ribosome binding site of the λ phage cII gene as shown in SEQ ID NO: 1 and SEQ ID NO: 2 or other ribosome binding site. Claims 39 and 44 have been amended to state that the suitable system for expressing heterologous proteins comprises the nucleic acid sequence of SEQ ID NO: 1 to more accurately reflect the relationship of the sequence components of SEQ ID NO: 1.

The Examiner further stated that claim 41 is redundant as dependent from claim 40 because enzymes are by definition useful for the biocatalysis of chemical reactions. Applicants submit that claim 41 is not redundant. Enzymes catalyze metabolic reactions in living organisms and can be used to catalyze substrate conversions in chemical reactions *in vitro*. Page 15, lines 8-25 and page 16, lines 1-7 of the specification discuss the methods of the invention and the uses of the proteins of interest. The term "chemical reactions" is used here to distinguish the use of the enzyme to catalyze reactions in living organisms from the use of enzymes to catalyze reactions *in vitro*, such as would be the case where the enzyme is added to a reaction mixture that contains the chemical substrates upon which the enzyme acts.

In view of the above, withdrawal of this entire section 112, second paragraph rejection is requested.

At page 7 of the Office Action, the Examiner rejected claims 21-24, 34, 35, 40 and 41 under 35 USC 102(b) as being anticipated by Cambiaghi *et al.*, U.S. Patent No. 5,424,196. The Examiner indicated that Cambiaghi *et al.* teaches expression of a heterologous enzyme, GI-7-ACA acylase in *E. coli* ATCC 9637.

Applicants traverse this rejection. Cambiaghi *et al.* discloses production of GI-7-ACA acylase in E.coli (ATCC accession number 9637) in Example 9. The gene encoding GI-7-ACA acylase is under the control of the tac promoter. Claim 21 has been amended to state that the suitable expression system comprises the P_{trp} promoter. Such a system is not disclosed in Cambiaghi *et al.* Cambiaghi *et al.* therefore does not anticipate claims 21-24, 34, 35, 40 and 41. Withdrawal of this 102(b) rejection is requested.

At page 8 of the Office Action the Examiner rejected claims 25-28, 33-38 and 43 under 35 USC 103(a) as being unpatentable over Cambiaghi *et al.* (U.S. Patent No.

5,424,196) in view of Makrides *et al.* (Microbiological Reviews 60: 512-538, 1996). The Examiner stated that Makrides *et al.* review strategies for high-level expression of genes in *E. coli* and teach the advantages of the P_{trp} promoter and fermentation strategies. Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to use optimal conditions for fermenting *E. coli* transformed with a DNA encoding a heterologous protein under control of P_{trp} because *E. coli* is the most studied and widely used host system for these purposes and the various conditions for its fermentation are known in the art. Absent unexpected results, various conditions are a matter of choice.

Applicants traverse this rejection.

Cambiaghi *et al.* is concerned with an enzymatic process for preparing 7-aminocephalosporic acid using a process that involves two enzymes, D-amino acid oxidase to convert Cephalosporin C into glutaryl-7-aminocephalosporanic acid and glutaryl-7-aminocephalosporanic acid acylase (Gl-7-ACA acylase) to deacylate the foregoing glutaryl derivative thereby converting it into 7-aminocephalosporanic acid. Cambiaghi *et al.* discloses production of Gl-7-ACA acylase in E.coli (ATCC accession number 9637) in Example 9. The gene encoding GI-7-ACA acylase is under the control of the tac promoter.

Makrides *et al.* is a review article that summarizes the scientific literature on gene expression in *E. coli*, and focuses on expression systems and experimental approaches useful for the overproduction of proteins. Makrides *et al.* discusses factors that influence the production of recombinant proteins in *E. coli* and discloses promoters, including P_{trp}, used for the high-level expression of genes in *E. coli* in Table 1. Page 514 of Makrides *et al.* states that the most widely used promoters for large-scale protein production use thermal incuction (λ_{PL}) or chemical inducers (trp). Makrides *et al.* discusses fermentation conditions generally at pages 525 and 526 and points out factors that can affect protein production.

Obviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching, suggestion or incentive supporting the combination. Citing references which merely indicate that isolated elements and/or

features recited in the claims are known is not a sufficient basis for concluding that the combination of claimed elements would have been obvious absent evidence of a motivating force which would impel persons skilled in the art to do what applicant has done.

It would not have been obvious to one skilled in the art to select the P_{trp} promoter as required by claim 38 (which has been canceled without prejudice and its limitation that the suitable system for expressing heterologous proteins comprises the P_{trp} promoter added to claim 21) and the fermentation conditions of claims 25-28 and 33-37 and substitute these for the promoter and conditions disclosed in Cambiaghi *et al.* to arrive at the claimed methods and host cells as asserted by the Examiner.

Cambiaghi *et al.* does not disclose or suggest the use of the P_{trp} promoter in conjunction with *E. coli* strain W for production of heterologous proteins.

Makrides *et al.* does not disclose *E. coli* strain W or suggest any promoters or fermentation conditions that would produce high levels of heterologous protein in that strain of *E. coli*. Makrides *et al.* discloses the P_{trp} promoter; however, the disclosure of the P_{trp} promoter is in a table with over 25 other promoters stated to be useful for high-level expression of genes in *E. coli* and the advantages of P_{trp} are not pointed out, let alone is there a suggestion that P_{trp} could be advantageously used in combination with strain W for production of heterologous proteins. Although fermentation conditions and factors affecting yield of heterologous protein are discussed in Makrides *et al.*, these are discussed generally, and specific fermentation conditions, such as the volume of the culture and composition of the culture medium are not disclosed. The use of tryptophan in the culture medium, as required by claims 26-28 and the use of a supplementary nitrogen source in the medium as required by claims 33-37 is not disclosed or suggested by Makrides *et al.* Thus, Makrides *et al.* does not provide adequate motivation or guidance for altering the method disclosed by Cambiaghi *et al.* in the manner asserted by the Examiner to obtain the claimed methods.

Applicants have found that *E. coli* strain W-containing expression systems comprising a P_{trp} promoter express heterologous proteins more effectively than *E. coli* strains DH5α or BL21. Examples 2 and 4 in the specification show that *E. coli* strain W

containing plasmids for expression of the nitrilase of *A. faecalis* or PAMII polyamidase, respectively, under the control of the P_{trp} promoter were more effective for expressing the protein.

There is nothing in Cambiaghi *et al.* or Makrides *et al.*, alone or in combination, which would cause a person of ordinary skill in the art to combine the selected teachings of each to arrive at the claimed process in the manner asserted by the Examiner. Accordingly, claims 25-28, 33-38 and 43 are not obvious over Cambiaghi *et al.* in view of Makrides *et al.* Withdrawal of this section 103 rejection is requested.

At page 9 of the Office Action, the Examiner rejected claims 29-32 under 35 USC 103(a) as being unpatentable over Cambiaghi *et al.* in view of Lee *et al.*, Biotechnology Letters 15(9): 971-974 (1993). The Examiner stated that it would have been obvious to one of ordinary skill in the art at the time the invention was made to use sucrose as a carbon source for optimal fermentation conditions of *E. coli* W.

Applicants traverse this rejection.

Claims 29-32 are drawn to the process of claim 21, wherein the suitable culture medium comprises sucrose as the main carbon source. Claim 21 has been amended to state that the suitable system for expressing a heterologous protein comprises a P_{trp} promoter.

Claims 29-32 are not obvious over Cambiaghi *et al* in view of Lee *et al.*

Cambiaghi *et al* has been discussed above.

Lee *et al.* discloses the use of sucrose as a carbon source for the high cell density cultivation of *E. coli* strain W, both wild-type and cells harboring a plasmid containing the *Alcaligenes eutrophus* PHA biosynthetic genes.

The combined disclosures of Cambiaghi *et al.* and Lee *et al.* fail to disclose or suggest the methods of claims 29-32. Neither of the cited references discloses the use of the P_{trp} promoter in conjunction with *E. coli* strain W for production of heterologous proteins. Accordingly, claims 29-32 are not obvious over Cambiaghi *et al.* in view of Lee *et al.* withdrawal of this section 103 rejection is requested.

At page 9 of the Office Action, the Examiner rejected claim 42 under 35 USC 103(a) as being unpatentable over Cambiaghi *et al.* in view of Petre *et al.* (U.S. Patent

No. 5,635,391). The Examiner stated that Petre *et al.* teaches expression of a heterologous nitrilase in *E. coli* under control of P_{trp} (claims 6-8) and the importance of nitrilase for conversion of nitriles to carboxylates (column 1, lines 19-37) and it would have been obvious to one of ordinary skill in the art to express nitrilase instead of Gl-7-ACA acylase in *E. coli* W.

Applicants traverse this rejection.

Claim 42 ultimately depends from claim 21 and is directed to the process wherein the heterologous protein is a nitrilase.

Claim 42 is not obvious over Cambiaghi *et al.* in view of Petre *et al.* As discussed above, obviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching, suggestion or incentive supporting the combination. Citing references which merely indicate that isolated elements and/or features recited in the claims are known is not a sufficient basis for concluding that the combination of claimed elements would have been obvious absent evidence of a motivating force which would impel persons skilled in the art to do what applicant has done.

There is nothing in Cambiaghi *et al.* or Petre *et al.*, alone or in combination, which would cause a person of ordinary skill in the art to combine the selected teachings of each to arrive at the claimed process in the manner asserted by the Examiner.

Cambiaghi *et al.* is concerned with an enzymatic process for preparing 7-aminocephalosporic acid using a process that involves two enzymes, D-amino acid oxidase to convert Cephalosporin C into glutaryl-7-aminocephalosporanic acid and glutaryl-7-aminocephalosporanic acid acylase (Gl-7-ACA acylase) to deacylate the foregoing glutaryl derivative thereby converting it into 7-aminocephalosporanic acid. Cambiaghi *et al.* discloses production of Gl-7-ACA acylase in E.coli (ATCC accession number 9637) in Example 9. The gene encoding GI-7-ACA acylase is under the control of the tac promoter. There is no suggestion in Cambiaghi *et al.* that a different enzyme could be substituted for the acylase, let alone that it could be substituted with a nitrilase.

Petre *et al.* disclose a nitrilase isolated from *Comamonas testosteron* and a DNA sequence encoding it. The nitrilase directly converts a nitrile to an ammonium

carboxylate. Petre *et al.* disclose that the enzyme is useful for industrial scale production of methionine. Petre *et al.* discloses expression of the nitrilase in *E. coli* strain TG1 under the control of the P_{trp} promoter (Example 5). Petre *et al.* discloses that the DNA sequence encoding the nitrilase can be expressed in microorganisms such as *E. coli*, but does not disclose *E. coli* strain W. There is also no disclosure or suggestion in Petre *et al.* to substitute the nitrilase with any other enzyme, let alone substitute it with an acylase.

There is thus no teaching, suggestion or incentive in the prior art supporting the selection of isolated features from Cambiaghi *et al.* and Petre *et al.* that have been used to retrospectively construct the method of claim 42 wherein a nitrilase is produced as the heterologous protein. The rejection of claim 42 over Cambiaghi *et al.* in view of Petre *et al.* is therefore improper and withdrawal of this rejection is requested.

In view of the above, the present application is believed to be in a condition ready for allowance. Reconsideration of the application is respectfully requested and an early Notice of Allowance is earnestly solicited.

Respectfully submitted,
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Marked Up Version Of Amended Claims

21. An industrial process for preparing a heterologous protein, comprising:

(1) introducing a suitable system for expressing heterologous proteins into an *E. coli* [bacteria] strain W host cell, wherein said suitable system for expressing heterologous proteins comprises a P_{trp} promoter; and

(2) seeding and culturing the *E. coli* [bacteria] strain W host cell in a suitable culture medium; such that the industrial process produces the heterologous protein.

22. The process of claim 21, wherein the *E. coli* [bacteria] strain W host cell is from the strain designated ATCC 9637.

23. The process of claim 21, wherein the *E. coli* [bacteria] strain W host cell is a derivative of the strain designated ATCC Number 9637 and is obtained by clonal selection or genetic manipulation.

39. The process of claim [38] 21, wherein the [P_{trp} promoter] suitable system for expressing heterologous proteins comprises the nucleic acid sequence of SEQ ID NO: 1.

43. An *E. coli* [bacteria] strain W host cell, comprising a suitable system for expressing heterologous proteins, wherein the suitable system comprises the P_{trp} promoter.

44. The *E. coli* [bacteria] strain W host cell of claim 43, wherein the [P_{trp} promoter] suitable system for expressing heterologous proteins comprises the nucleic acid sequence of SEQ ID NO: 1.